Improving Methods for Analyzing Antimalarial Drug Efficacy Trials: Molecular Correction Based on Length-Polymorphic Markers *msp-1*, *msp-2*, and *glurp*

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Malaria

- Endemic in over 100 countries and causes ~400,000 deaths per annum
- Prompt treatment is an essential and effective public-health tool but drug resistance poses a constant threat
- Drug efficacy trials monitor the continued efficacy of front-line drugs against falciparum malaria
  - Over-estimates = countries retaining a failing drug as first-line treatment with associated increases in morbidity and mortality
  - Under-estimates = removal of an effective treatment with substantial practical and economic implications
- Trials are challenging
  - Require long durations of follow-up to detect drug failures
  - Patients are frequently re-infected during follow up
Mechanistic PK-PD model for malaria

Patient PK
- Absorption rate
- Volume of distribution
- Clearance

Parasite PD
- Maximal effect
- IC50
- Slope factor

PK/PD model:
\[
\frac{dP}{dt} = P \left( a - f(I) - \sum_{i=1}^{n} f(C_i) \right)
\]

Individual treatment outcome

% cured population level

Multiple PK models
- Add variability

Individual treatment outcome
- Infection cleared
- Infection not cleared

Modifications for clinical trial analysis

Patient PK

- Absorption rate
- Volume of distribution
- Clearance

PK/PD model

\[
\frac{dP}{dt} = P \cdot \left( a - f(t) - \sum_{i=1}^{n} f(C_i) \right)
\]

Parasite PD

- Maximal effect
- IC50
- Slope factor

Individual treatment outcome

- Drug concentration
- Detection limit
- Parasites emerging from the liver

Modifications

- Multiple clones at the time of treatment
- Reinfections emerge from the liver at a rate reflecting local malaria intensity
- Parasitemia of each clone was tracked and updated each day to reflect
  1. Extent of drug killing based on the PKPD
  2. Growth rate of each clone
- Each clone assigned a genetic profile based on 3 specific molecular markers

Major challenge of efficacy trials

- Distinguishing reason for recurrent infection i.e. recrudescent versus new infection

Advantage of R

- Free, open-source software that is readily available to researchers in developing countries


Clinical trial simulations

**PK-PD Model**: tracks the genotype of all existing and new malaria clones over time and allows us to determine the true drug failure rate.

**Reality**: not all genetic signals will be observed in every blood sample, detectability varies based on relative frequency, allelic length and family.

**Simulations**: incorporated these problems to generate the genetic signals that would be observed in samples and investigated the predictive capability of four molecular correction algorithms.
Results – comparing algorithms

WHO method

No glup

Patient classified as
Recrudescence
Reinfection

- WHO algorithm correctly classifies nearly all reinfections, but misclassifies around one-third of recrudescences
- No-glup algorithm is similar to the WHO algorithm
- >= 2/3 markers algorithm had fewer misclassifications and was also more balanced
- Allelic family switch algorithm correctly classifies a large proportion of recrudescences but misclassifies a large number of reinfections.

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## Results – re-analysis of clinical trial data

<table>
<thead>
<tr>
<th>Country</th>
<th>Drug</th>
<th>Molecular assignment</th>
<th>WHO</th>
<th>No glurp</th>
<th>≥ 2/3 markers</th>
<th>Allelic family switch</th>
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</thead>
<tbody>
<tr>
<td>Rwanda</td>
<td>Artemether -</td>
<td>Recrudescence</td>
<td>17</td>
<td>27</td>
<td>36</td>
<td>59</td>
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<tr>
<td></td>
<td>Lumefantrine</td>
<td>New infections</td>
<td>93</td>
<td>83</td>
<td>73</td>
<td>51</td>
</tr>
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<td>DHA - Piperquine</td>
<td>Recrudescence</td>
<td>3</td>
<td>6</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>New infections</td>
<td>40</td>
<td>37</td>
<td>35</td>
<td>25</td>
</tr>
</tbody>
</table>

- Model predictions using the WHO method were highly consistent with existing in vivo data
- Predictions with the newly proposed “≥ 2/3 markers” algorithm suggest the WHO-method under-estimates true treatment failure rates
Summary

• Model was applied to multiple drugs across a range of transmission settings and was able to quantify the accuracy of failure rate estimates in therapeutic efficacy studies.

• Accurately predicting failure estimates is clinically important.
  • Molecular correction is essential to avoid substantial over-estimates of failure rates.
  • Current WHO-recommended algorithm consistently under-estimates the true failure rate.
  • A newly-proposed algorithm (“≥ 2/3 markers”) produces accurate failure rate estimates, robust at all levels of transmission intensity.
  • Long durations of patient follow-up may be counterproductive; large numbers of new infections accumulate and may be misclassified, over-estimating drug failure rate.
Summary

• Implemented in R - mainly base R, with *dplyr*, *ggplot*, *survival* and *survminer* packages for calculating drug efficacy

• Open source software particularly attractive for tropical diseases as they typically occur in countries with limited resources and paying for licenses can be an unnecessary barrier to progress

• Future
  • Developing a Bayesian algorithm to more accurately predict failure rates
  • Developing an R package that could be used to process user sample data and return failure rate estimates based on all the algorithms
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